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(54) Title: PREPARATIONS CONTAINING Asp^{B28} HUMAN INSULIN AND NICOTINAMIDE

(57) Abstract

Preparations containing Asp^{B28} human insulin and nicotinamide or a salt thereof show superior pharmacological properties.

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PREPARATIONS CONTAINING Asp^{B28} HUMAN IN INSULIN AND NICOTINAMIDE.

The present invention relates to insulin preparations containing Asp^{B28} human insulin and nicotinamide or a salt thereof. The preparations exhibit interesting therapeutic properties.

BACKGROUND OF THIS INVENTION

For decades, insulin has been used for the treatment of diabetics. Among the commercially available insulin preparations, rapidly acting, intermediately acting and prolonged acting preparations can be mentioned. Examples of rapidly acting preparations are neutral solutions of zinc containing insulin which, inter alia, are known under the trade marks Actrapid® and Velosulin®. Faster absorption of insulin compared to that obtained with the last-mentioned preparations can be effected by using monomeric or dimeric insulin analogs, vide European patent application No. 86306721, or by formulating insulin with magnesium salts, vide European patent application No. 87309229.

According to Diabetic Medicine 6 (1989), 568, diabetic patients have been treated orally with nicotinamide and they have been injected with insulin. The object of the study was to investigate whether small oral doses of nicotinamide would improve metabolic control. In this known study, nicotinamide and insulin was not mixed before administration. A similar study is described in Diabetologia 32 (1989), 160.

A composition for application to the hair and scalp which may contain nicotinamide and/or isophane insulin has been suggested in British patent No. 1,603,639. No compositions containing both nicotinamide and isophane insulin are described therein. This composition is for use in stimulating hair growth and in treating alopecia and excessive hair loss.

International Patent Application No. WO 91/09617 relates to insulin preparations containing nicotinamide or nicotinic acid or a salt thereof and insulin

or an insulin derivative. In this known application, there is no mentioning of Asp^{B28} human insulin. Compared with this known invention, the present invention is a so-called selection invention.

BRIEF DISCUSSION OF THIS INVENTION

According to this invention, it has, surprisingly, been found that preparations containing Asp^{B28} human insulin and nicotinamide or a salt thereof have superior properties. The superiority of such preparations is, for example, the very rapid onset of insulin action when such preparations are administered to humans.

Thus, the present invention relates to preparations containing Asp^{B28} human insulin and nicotinamide or a salt thereof. Such preparations can be used for parenteral administration to diabetics.

The preparations of this invention may, if desired, furthermore contain precipitated insulin or a precipitated insulin derivative having protracted action. By adding such precipitates, a suspension is obtained.

ATTRIBUTES OF THIS INVENTION

Absent retarding substances in the insulin preparations of this invention, the absorption of Asp^{B28} human insulin was surprisingly found to be faster than that of the reference insulin used in the example below. This property is useful for a rapidly acting insulin, in particular in connection with a multiple injection regimen where insulin is given before each meal. With quicker onset of action, the insulin can conveniently be taken closer to the meal than with conventional rapidly acting insulin solutions. Furthermore, a faster disappearance of insulin probably diminishes the risk of post meal hypoglycemia.

As an example, the preparations of this invention are believed to be well suited for application in fountain pen like devices used for multiple injection

insulin therapy.

DETAILED DISCUSSION OF THIS INVENTION

Preferably, the preparations according to this invention containing Asp^{B28} human insulin and nicotinamide or a salt thereof are present as a solution thereof, preferably in an aqueous solution.

Preferably, Asp^{B28} human insulin of high purity is used.

The content of Asp^{B28} human insulin in solutions of this invention may be in the range of 20 to 500 international units (IU) per ml, preferably in the range of 40 to 100 IU/ml, in preparations for injection. However, for other purposes of parenteral administration, the content of Asp^{B28} human insulin may be higher. The solution of Asp^{B28} human insulin may be mixed with a solid insulin material such as zinc insulin crystals or zinc protamine insulin crystals.

According to this invention, the preferred concentration of nicotinamide plus salts thereof is in the range from about 0.01 to about 1 M, preferably from about 0.05 to about 0.5 M.

Known to the art stabilizers and preservatives may be present in the insulin preparations of this invention.

The preservative present in the insulin preparation of this invention may be as in the heretofore conventional insulin preparations, for example phenol, m-cresol and methylparaben.

As an example, the preparations of this invention have a pH value in the range from about 3 to about 8.5.

For the preparation of aqueous insulin preparations according to this invention, a slightly acidic solution of Asp^{B28} human insulin can be mixed with a solution containing all the other components of th final preparation. Then follows adjustment of pH value, if required, stirring until a clear solution is obtained and finally sterile filtration. If desired, a sterile, protracted acting insulin

suspension may be added to the sterile insulin solution yielding a preparation with biphasic action. In order to protect the preparations from the denaturation that may take place by occasional heating and shaking, known stabilising agents, such as phospholipids, may be included.

If the composition of this invention also contains precipitated insulin or a precipitated insulin derivative, a biphasic preparation may be obtained. Compared with the known biphasic insulin preparations, the biphasic preparations according to this invention have a more rapid onset of blood sugar lowering effect. Examples of precipitated insulin are zinc insulin crystals and protamine zinc insulin crystals.

The insulin preparations of this invention can be used in the treatment of diabetics by parenteral administration. It is recommended that the dosage of the insulin preparations of this invention which is to be administered to the patient be selected by a physician similarly to the selection of the dosage of known insulin preparations for injection to human beings.

This invention is further illustrated in the following examples which, however, are not to be construed as limiting.

Absorption studies

The experiments described in the following examples were performed as absorption studies in pigs. Test and reference preparations (all solutions) were made from ^{125}I-labelled human insulin or Asp^{B28} human insulin derivative. 6 IU (0.036 μmol) of the test preparation was injected at one side of the neck and 6 IU of the reference preparation at the other side in each of a number of pigs. The absorption was followed by external monitoring of the radioactivity remaining at the site of injection. The injections were performed by NovoPen^{TM}, using a normal needle, inserted to a depth of 5 mm (subcutaneously).

Terms used in the examples

Zn^{++}/hexamer:	Number of zinc ions per insulin hexamer.
$T_{75\%}$:	Time until 75% of initial radioactivity remaining.
$T_{50\%}$:	Time until 50% of initial radioactivity remaining.
$F_{75\%}$:	$T_{75\%}$ (test)/$T_{75\%}$ (reference).
$F_{50\%}$:	$T_{50\%}$ (test)/$T_{50\%}$ (reference).

Example 1

125-I-labelled Asp^{B28} human insulin was used for the absorption study. 0.06 ml of a reference solution (0.6 mmol/l Asp^{B28}, 3 Zn/hexamer, 16 g/l glycerol, 3 g/l phenol, pH value 7.4) and 0.06 ml of test solution, prepared by substituting 0.26 mol/l nicotinamide for glycerol, were injected contralaterally in the neck of 6 pigs followed by counting over the injection sites. A cross-over was made 6 days after. The $T_{75\%}$ averages (± SEM) were: 21 ± 1 and 36 ± 4 minutes for test and reference, respectively ($p < 0.01$). The $T_{50\%}$ averages (± SEM) were 52 ± 3 and 68 ± 5 minutes, respectively ($p < 0.02$). In a cross-over study in four fasted pigs (mean weight 103 kg), each pig was injected with 0.06 ml of the test solution on one day and 0.06 ml of the reference solution on the other day. Plasma glucose was measured at various times after injection.

The results (minutes/mean decrease in mmol/l glucose for test/reference) were:

Time after injection Minutes	Decrease in plasma glucose	Decrease in plasma glucose Reference, mmol/l
0	0	0
20	2.3	0.9
40	2.6	2.4
60	2.7	2.4
80	2.4	2.5
100	2.4	2.6
120	2.5	2.5
150	1.8	2.3
180	1.4	2.1
210	1.3	1.7
240	1.0	1.4
270	0.7	1.1
300	0.5	0.8

Based upon these results, it can be concluded that formulation of Asp^{B28} human insulin with nicotinamide results in initially faster absorption of Asp^{B28} human insulin after subcutaneous injection in pigs and earlier onset of action. The monomeric insulin analog formulated with nicotinamide may therefore be more appropriate as an ultra short acting preparation, given just before meals, than the analog in conventional formulation.

CLAIMS

1. Insulin preparations for injection or infusion, characterized in that it comprises Asp^{B28} human insulin and nicotinamide or a salt thereof.

2. Preparation according to Claim 1, characterized in that the content of nicotinamide plus salts thereof is in the range from about 0.01 to about 1 M, preferably from about 0.05 to about 0.5 M.

3. Preparation according to any one of the preceding claims, characterized in that the preparation is to be used as a preparation for the treatment of diabetics.

4. Preparation according to any one of the preceding claims, characterized in that it, additionally, comprises zinc ions, preferably less than about 6 zinc ions per hexamer insulin or insulin derivative, more preferred less than about 4 zinc ions per hexamer insulin or insulin derivative.

5. Preparation according to any one of the preceding claims, characterized in that the pH value is above about 3, preferably in the range from about 5 to about 8.5, more preferred from about 6 to about 8, most preferred from about 6.5 to about 7.5.

6. Preparation according to any one of the preceding claims, characterized in that it has an activity in the range below about 500 IU per ml, preferably from about 20 to 200 IU per ml, most preferred from about 40 to about 150 IU per ml.

7. Preparation according to any one of the preceding claims, characterized in that it is rapidly acting.

8. The use as a preparation for the treatment of diabetics with a preparation comprising Asp^{B28} human insulin and nicotinamide or a salt thereof.

9. Any novel feature or combination of features described herein.

Novo Nordisk A/S

5 1995-09-22, ToN/KGF 4182.204-WO

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 95/00395

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: A61K 38/28, A61K 31/465
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPIL, CLAIMS, JAPIO, CA, BIOSIS, MEDLINE, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 9109617 A1 (NOVO NORDISK A/S), 11 July 1991 (11.07.91), see e.g. claims	1-9
Y	--	1-9
Y	EP 0214826 A2 (NOVO INDUSTRI A/S), 18 March 1987 (18.03.87), see Example 9 and abstract	1-9
	--	
A	WO 9007522 A1 (NOVO-NORDISK A/S), 12 July 1990 (12.07.90), see e.g. page 10, line 19	1-9
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[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" erlier document but published on or after the international filing date
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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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Date of the actual completion of the international search	Date of mailing of the international search report
4 January 1996	15 -01- 1996
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Carolina Gómez Lagerlöf Telephone No. +46 8 782 25 00

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 95/00395

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	GB 1603639 A (HELEN FREDA HAGGAR), 25 November 1981 (25.11.81), whole document, especially page 1	1-9

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 95/00395

Box I **Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 9
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

The claim do not define the matter for which protection is sought.
The claim is not clear and concise. (See art. 6).

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II **Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark n Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/DK 95/00395

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A1- 9109617	11/07/91	AT-T-	122567	15/06/95
		AU-B-	641721	30/09/93
		AU-A-	7047191	24/07/91
		CA-A-	2070814	22/06/91
		DE-D,T-	69019534	21/09/95
		EP-A,B-	0506792	07/10/92
		SE-T3-	0506792	
		ES-T-	2072596	16/07/95
		US-A-	5382574	17/01/95
EP-A2- 0214826	18/03/87	SE-T3-	0214826	
		AT-T-	113061	15/11/94
		AU-B,B-	593274	08/02/90
		AU-A-	6206686	05/03/87
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		DE-D,T-	3650101	23/02/95
		JP-A-	62053999	09/03/87
		KR-B-	9400756	29/01/94
		NO-B,C-	177009	27/03/95
WO-A1- 9007522	12/07/90	AU-B-	641631	30/09/93
		AU-A-	4834490	01/08/90
		CA-A-	2006578	23/06/90
		EP-A,A,A	0375437	27/06/90
		JP-T-	4502465	07/05/92
		US-A-	5164366	17/11/92
GB-A- 1603639	25/11/81	NONE		